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Low Density Lipoprotein Particle Size and Coronary Artery Disease

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Decreased plasma low density lipoprotein (LDL) particle size has been associated with premature coronary artery disease (CAD). We examined LDL particle size by 2–16% gradient gel electrophoresis in 275 men with CAD (>75% cross-sectional-area stenosis) and 822 controls. Seven major LDL size bands (with LDL-1 [$d=1.025$ – 1.033 g/ml] being the largest and LDL-7 [$d=1.050$ – 1.063 g/ml, the smallest]) were identified. Because most subjects had two or more adjacent LDL bands, an LDL score was calculated for each subject, with the relative area in each band taken into consideration. Four major LDL particle size groups were classified in the present studies: large LDL, intermediate LDL, small LDL, and very small LDL. The use of β -blockers was significantly associated with smaller LDL particles. After adjusting for use of this medication, small LDL particles were still more prevalent in CAD patients (39%) compared with controls (27%). The prevalence of large LDL particles was lower in CAD patients (3%) than in controls (24%). Intermediate LDL particles were the most prevalent in both groups, 49% in CAD patients and 46% in controls. The difference in LDL particle size between CAD patients and controls was not independent but was highly associated ($p<0.0001$) with elevated triglyceride levels and decreased high density lipoprotein (HDL) cholesterol levels. Significantly higher LDL cholesterol levels were found in subjects with intermediate and small LDL particles than in those with large or very small LDL particles. In addition, CAD patients with intermediate or small LDL particles had significantly ($p<0.01$) lower HDL cholesterol and apolipoprotein A-I levels and higher LDL cholesterol levels than did controls in the same group. Smoking, hypertension, diabetes, and HDL and LDL cholesterol levels were strong discriminators between CAD patients and controls, while triglycerides and LDL particle size did not add significant information to the model. These data indicate that small LDL particle size is not an independent discriminator for CAD after conventional risk factors and lipoprotein parameters such as LDL and HDL cholesterol have been taken into account. (*Arteriosclerosis and Thrombosis* 1992;12:187–195)

Elevated plasma levels of low density lipoprotein (LDL) cholesterol are associated with increased risk of developing coronary artery disease (CAD).^{1–3} LDL particles are the major cholesterol-carrying lipoproteins in plasma, and they

consist of a hydrophobic core of cholesterol esters and triglycerides surrounded by phospholipids, free cholesterol, and one molecule of apolipoprotein (apo) B-100 on the surface of the particle.⁴ Apo B is a glycosylated protein of apparent molecular weight of 550 kd and provides structural integrity for the LDL particle. Apo B is the ligand for the LDL receptor that allows for the catabolism of LDL particles by receptor-mediated endocytosis.⁵

LDL particles vary in size and hydrated density.^{6,7} On gradient gel electrophoresis, seven LDL subfractions can be identified.^{6,8,9} The factors associated with decreased LDL particle size are male gender, elevated triglyceride, and decreased high density lipoprotein (HDL) cholesterol levels.^{9,10} In addition, the presence of small LDL particles has been associated with low-fat and high-carbohydrate diets.¹¹ In vitro experiments suggest that triglyceride-rich, cholesterol ester-depleted LDL particles have a de-

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creased affinity for the LDL receptor compared with normal LDL.¹²

Apo B-rich LDL particles have been associated with CAD, even in subjects with normal lipid levels. In patients with the disorder hyperapobetalipoproteinemia, LDL particles are smaller and denser than LDL in normal subjects. In these patients, LDL apo B in plasma is elevated despite normal LDL cholesterol levels. The number of LDL particles is therefore increased.¹³

Recent studies have indicated that low-molecular-weight LDL particles are more prevalent in men with CAD than in controls and that small LDL particles (<255 Å) have been associated with a threefold increased risk of myocardial infarction.^{14,15} These differences are no longer significant when adjustments for triglyceride levels are made. Because of the interrelations of LDL particle size with lipid and lipoprotein levels, particularly triglyceride and HDL cholesterol levels, and the use of medications,¹⁶ the role of LDL particle size in patients with CAD is uncertain. In the present study, we compared LDL particle size in men with CAD and in controls in relation to lipids, lipoproteins, apo A-I and B, and medication use. We also examined these biochemical parameters in the presence of smoking, hypertension, and diabetes. Our study confirms previous studies on LDL particle size and CAD. The association between LDL particle size and CAD is not independent when established cardiovascular risk factors have been considered.

Methods

Study Subjects

Coronary artery disease patients. Patients ($n=280$) with clinical evidence of CAD underwent elective cardiac catheterization for the diagnosis and extent of CAD at the New England Medical Center Hospital, Boston, Mass. Blood samples were drawn before catheterization and after at least a 12-hour fast. Patients were referred mainly from the greater Boston area and eastern Massachusetts. All subjects were Caucasian men below 60 years of age (mean \pm SD, 50 ± 7 years) at the time of their coronary angiography. Patients with acute myocardial infarction, surgery, or trauma in the preceding 6 weeks before admission were not included. Information on medications (diuretics, β -blockers, and calcium channel-blocking drugs) was obtained by direct interview and review of the patients' medical charts. Patients were coded as not taking medications if they had been off the drug for at least 4 weeks. Only patients who had been taking β -blockers were noted to have effects on lipoproteins and LDL size; therefore, adjustments had to be made for their effect. Hypertensives were identified as those individuals taking antihypertensive medications and/or those having a diastolic blood pressure >95 mm Hg. Smokers were those who smoked more than 10 cigarettes per day. Diabetics were defined as those individuals on hypo-

glycemic medication and/or whose fasting glucose levels were >140 mg/dl. The degree of CAD was determined by two independent cardiologists who were unaware of the patient's inclusion in the study. The presence of CAD was defined as greater than 50% stenosis of a major coronary artery on multiple projections ($>75\%$ cross-sectional-area stenosis). Patients with triglyceride levels ≥ 500 mg/dl were not included in this analysis ($n=5$). The final sample size was 275 patients ($n=96$ off β -blocker medication, $n=179$ on β -blocker medication). Other medications were not considered because no significant association with lipid parameters was noted among patients with CAD.

Controls. Men ($n=822$) aged 40–60 years old (mean \pm SD, 49 ± 6 years) from the offspring cohort of the Framingham Heart Study were selected as controls.² Subjects with clinical manifestations of cerebrovascular, peripheral vascular, or coronary artery disease; a history of myocardial infarction; use of medications known to affect lipids; or with triglyceride levels ≥ 500 mg/dl were not included. The criteria to identify smokers, diabetics, and hypertensives were the same as described above for the CAD patients. The use of a free-living population as a control group was selected because the presence of patients with clean arteries was rare in the CAD population, and these patients usually go to the hospital because they have other health complications that may affect the parameters of interest. The control subjects in this study represent randomly selected healthy Caucasian men who served as more appropriate controls.

Lipid, Lipoprotein, Apolipoprotein, and Low Density Lipoprotein Particle Size Determinations

Blood was drawn from subjects after a 12-hour fast into tubes containing EDTA. Plasma was separated after centrifugation at 2,500 rpm for 20 minutes at 4°C. Plasma total cholesterol, triglyceride, and HDL cholesterol levels were determined enzymatically on an Abbott Diagnostics ABA-200 bichromatic analyzer. The HDL supernate was obtained after precipitation of apo B-containing lipoproteins with dextran- Mg^{2+} .¹⁷ LDL cholesterol was calculated as described by Friedewald et al,¹⁸ unless the triglyceride concentration was above 400 mg/dl, in which case cholesterol was measured in the $d<1.006$ g/ml infranate after ultracentrifugation. LDL cholesterol was then calculated as infranate cholesterol minus HDL cholesterol.¹⁹ Plasma apo A-I and apo B were determined by a noncompetitive enzyme-linked immunosorbent assay (ELISA) as previously described.²⁰ To compensate for hospital effect, HDL cholesterol was adjusted by a factor of 1.0916 and apo A-I by a factor of 1.101.²¹

LDL subfractions were separated by 2–16% gradient gel electrophoresis (PAA 2–16%, Pharmacia, Piscataway, N.J.) as previously described.⁹ All gels included a characterized pooled plasma standard. Scanning was performed on an LKB Ultrascan XL

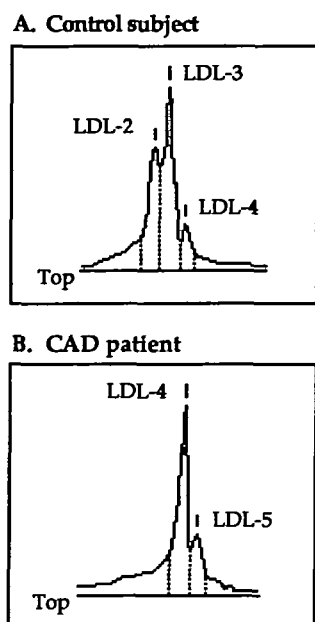


FIGURE 1. Low density lipoprotein (LDL) distribution scan as determined by 2–16% gradient gel electrophoresis in (panel A) a control subject with a predominant LDL-3 band (51% of area) and two adjacent bands, LDL-2 (34% of area) and LDL-4 (15% of area). LDL particle score is 2.81, or $(2 \times 0.34) + (3 \times 0.51) + (4 \times 0.15)$. Panel B: LDL scan for a coronary artery disease patient with a predominant LDL-4 band (81% of area) and an adjacent LDL-5 band (19% of area). LDL particle score is 4.19, or $(4 \times 0.81) + (5 \times 0.19)$.

laser densitometer (LKB Instruments Inc., Paramus, N.J.) interfaced with an AT&T personal computer (LKB) and a Canon PJ-108A printer using the LKB GSXL software for peak integration. Each subject was assigned an LDL size, with the largest, LDL-1, being found in the density range 1.025–1.033 g/ml; LDL-2 and LDL-3 in the range 1.033–1.038 g/ml; LDL-4 and LDL-5 in the range 1.038–1.050 g/ml; and the smallest, LDL-6 and -7, being found in the range 1.050–1.063 g/ml. Because we found that 30% of subjects had adjacent secondary bands of at least 30% of the total band area and that 88% of subjects have one major adjacent band and one or two minor adjacent bands,⁹ we assigned each subject an LDL particle score to account for all the bands present. The LDL particle score for each subject was calculated as the sum of the relative areas under all the LDL bands present. For example, Figure 1 shows the LDL particle size scan of a control subject and a patient with CAD. The control subject has a predominant LDL-3 band (51% of area) and two adjacent bands, LDL-2 (34% of area) and LDL-4 (15% of area), so the LDL particle score is 2.81, or $(2 \times 0.34) + (3 \times 0.51) + (4 \times 0.15)$. The CAD patient has a predominant LDL-4 band (81% of area) and an adjacent LDL band, LDL-5 (19% of area); thus, the calculated LDL particle score is 4.19, or $(4 \times 0.81) + (5 \times 0.19)$. A higher LDL particle score corresponds to a smaller, denser LDL particle.

In addition, we classified subjects in this study into four LDL particle score groups. The LDL score groups were previously defined according to the population distribution observed in the 822 subjects in the control group. The groups were defined as 1) large-LDL particle score group (LDL score ≥ 1.00 and ≤ 2.60); 2) intermediate-LDL score group (LDL score > 2.60 and ≤ 3.80); 3) small-LDL score group (LDL score > 3.80 and ≤ 5.60); and 4) very-small-LDL score group (LDL score > 5.60). These LDL score groups were chosen because they represent naturally occurring clusters of LDL particle scores in a randomly selected normal population. Finally, for ease of interpretation and comparison with other previous reports, we estimated the angstrom equivalent for the LDL particle score in each group (R.M. Krauss, personal communication). The four LDL particle score groups reported in this study correspond to the following ranges: 1) large-LDL particle score group (≥ 267 Å); 2) intermediate-LDL particle score group (≤ 266 Å and ≥ 260 Å); 3) small-LDL particle score group (< 260 Å and ≥ 248 Å); and 4) very-small-LDL particle score group (< 248 Å).

Statistical Analysis

Statistical analyses were performed with Statistical Analysis Systems software (SAS, Cary, N.C.). The procedures used included *t* test analysis for mean comparisons of lipoprotein and apolipoprotein plasma parameters between CAD patients on and off β -blockers and controls. Because CAD patients on and off medication were significantly different from each other in most plasma parameters, we carried out all the subsequent comparisons by using regression adjustments for the use of β -blockers. The general linear model procedure was used for the LDL particle score analysis of covariance and three-way analysis of variance. The LDL particle score distribution plots and Pearson or Spearman correlation coefficients were carried out using the Chart and Corr procedures, respectively, in the SAS system. Stepwise discriminant analyses with backward and forward elimination procedures were used to identify plasma parameters that discriminate men with CAD from controls. Of the biochemical parameters, HDL cholesterol and apo A-I levels and LDL cholesterol and apo B levels are highly intercorrelated, $r=0.72$ and $r=0.67$, respectively. Thus, we used two separate models. In the first one we included HDL and LDL cholesterol, but not apo A-I and apo B levels, and in the second one, we included apo A-I and apo B levels but not HDL and LDL cholesterol.

Results

Plasma Lipids, Lipoproteins, Apolipoproteins, and Low Density Lipoprotein Particle Score in Coronary Artery Disease Cases and Controls

Mean plasma lipoprotein and apolipoprotein concentrations and LDL particle score for men with CAD on and off β -blockers and controls are given in Table 1. Overall, men with CAD had significantly

TABLE 1. Lipoprotein and Apolipoprotein Levels in Men With Coronary Artery Disease and Controls

Parameter (mg/dl)	Controls (n=822)	Coronary artery disease			p value		
		All (n=275)	Off β -blockers (n=96)	On β -blockers (n=179)	Control vs. all CAD	Control vs. CAD off β -blockers	CAD off vs. CAD on β -blockers
Total cholesterol	213 \pm 37	212 \pm 49	222 \pm 55	207 \pm 44	0.9	0.1	0.02
Triglycerides	132 \pm 81	186 \pm 84	174 \pm 83	192 \pm 85	<0.0001	<0.0001	0.09
LDL cholesterol	141 \pm 34	143 \pm 46	153 \pm 54	138 \pm 40	0.5	0.03	0.01
HDL cholesterol*	45 \pm 12	35 \pm 10	37 \pm 12	33 \pm 8	<0.0001	<0.0001	0.01
Apo B	97 \pm 29	108 \pm 29	111 \pm 29	107 \pm 29	<0.0001	<0.0001	0.2
Apo A-I*	136 \pm 32	110 \pm 26	115 \pm 28	108 \pm 24	<0.0001	<0.0001	0.03
LDL particle score†	3.37 \pm 1.10	4.32 \pm 1.20	4.02 \pm 1.10	4.48 \pm 1.30	<0.0001	<0.0001	0.002

CAD, coronary artery disease; LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein.

*HDL increased from 32 \pm 10 to 35 \pm 11 (\times 1.0916), and apo A-I increased from 100 \pm 22 to 111 \pm 25 (\times 1.101) to compensate for hospital effect (see Reference 21).

†LDL particle score for each subject was calculated by multiplying each LDL band present by its percent relative area (see text for details).

higher triglyceride and apo B levels, lower HDL cholesterol and apo A-I concentrations, and smaller LDL particles (represented by a higher LDL particle score). When CAD patients taking β -blockers were compared with those not taking this medication, men with CAD on β -blockers had significantly lower total, LDL, and HDL cholesterol and apo A-I concentrations as well as significantly smaller LDL particles than did CAD patients off β -blockers. When only those CAD patients not taking β -blockers were compared with controls, the magnitude of the difference was reduced for HDL cholesterol, apo A-I, and particularly for triglyceride levels and LDL particle score. However, the magnitude of the difference increased for LDL cholesterol, from 1% to 8%, and a significant difference ($p<0.05$) was found in LDL cholesterol between CAD patients off β -blockers and controls. All the subsequent analyses were adjusted for the use of β -blockers.

Associations Between Plasma Parameters and Low Density Lipoprotein Particle Score

Table 2 shows the Pearson or Spearman correlation coefficients in the CAD patients on and off β -blockers and in controls. Smaller LDL particles in the three groups were associated with increased triglyceride levels and decreased HDL cholesterol and apo A-I levels. Smaller LDL particles were also associated with increased diabetes, hypertension, total cholesterol, and apo B levels in controls and with apo B levels in CAD patients off β -blockers.

A comparison of LDL particle score in CAD patients and controls, after adjusting for significant covariates, is shown in Table 3. When triglyceride levels or HDL cholesterol levels alone were entered, the differences between CAD patients and controls remained significant ($p<0.04$). Adjusting for triglyceride and HDL together significantly reduced the differences in LDL particle score between CAD patients and controls so that the differences were no longer significant. The addition of other significant biochemical covariates did not change the magnitude

of this difference in LDL particle score between CAD patients and controls.

Population Distribution of Low Density Lipoprotein Particle Score in Coronary Artery Disease Cases and Controls

The population distributions of LDL particle score in CAD patients on and off β -blockers and controls are shown in Figure 2. These data show four major LDL particle score groups, with different frequencies in the three groups studied. Large LDL particles were found more frequently in the control group (24%), with almost no CAD patients off or on β -blockers having these large particles. Normal men and CAD patients were more likely to have the intermediate LDL particles; however, these intermediate particles were less prevalent in CAD patients on β -blockers. In contrast, 39% of CAD patients off

TABLE 2. Univariate Correlation Coefficients Between Low Density Lipoprotein Particle Score* and Lipoprotein Parameters in Men With Coronary Artery Disease and Controls

Parameter	Controls (n=822)	CAD	
		off β -blockers (n=96)	on β -blockers (n=179)
Age	-0.05	-0.07	-0.04
Diabetes	0.08†	-0.08	-0.02
Hypertension	0.13§	0.15	0.09
Smoking habits	0.00	0.01	-0.06
Triglyceride	0.73§	0.67§	0.52§
Total cholesterol	0.22§	0.06	0.03
LDL cholesterol	0.08	-0.06	-0.11
HDL cholesterol	-0.57§	-0.41§	-0.41§
Apo A-I	-0.32§	-0.32‡	-0.17†
Apo B	0.40§	0.32‡	0.09

CAD, coronary artery disease; LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein.

*LDL particle score for each subject was calculated by multiplying each LDL band present by its percent relative area (see text for details).

† $p<0.01$, ‡ $p<0.001$, § $p<0.0001$.

TABLE 3. Lipoprotein-Adjusted Low Density Lipoprotein Particle Score* Means in Men With Coronary Artery Disease and Controls

Covariates	Adjusted LDL particle score*		p value
	Controls (n=822)	CAD (n=275)	
None	3.37±0.04	4.32±0.11	<0.0001
β-Blocker use†	3.44±0.05	4.09±0.10	<0.0001
β-Blocker use† and triglycerides‡	3.55±0.03	3.78±0.07	0.01
β-Blocker use§ and HDL cholesterol‡	3.41±0.03	3.64±0.10	0.04
β-Blocker use§, triglycerides‡, and HDL cholesterol‡	3.59±0.03	3.65±0.08	0.5

LDL, low density lipoprotein; CAD, coronary artery disease; HDL, high density lipoprotein.

*LDL particle score for each subject was calculated by multiplying each LDL band present by its percent relative area (see text for details). Values are given as mean±SEM.

Significant covariate † $p<0.01$, ‡ $p<0.0001$, § $p<0.05$. No other covariates were significant when triglyceride and HDL cholesterol were adjusted for.

β-blockers and 45% of patients on β-blockers had small LDL particles versus 27% in controls. Very small LDL particles were rarely found among the control subjects. CAD patients on β-blockers had the highest prevalence of very small LDL particles. In sum, CAD subjects are characterized by a lack of large LDL particles and an increased prevalence of small LDL particles compared with controls.

Triglyceride, High Density Lipoprotein Cholesterol, Apolipoprotein A-I, and Apolipoprotein B Levels in Four Major Low Density Lipoprotein Particle Score Groups in Coronary Artery Disease Cases and Controls

The plasma parameters, adjusted for the use of β-blockers, in the four major LDL particle score groups in CAD patients and controls are shown in Table 4. By three-way analysis of variance, the associations of all the plasma parameters with LDL score groups and with disease status (CAD or controls) were significant ($p<0.01$). In this model, the use of β-blockers was associated with decreased LDL cholesterol and apo B levels. Triglyceride levels were lower in the large-LDL particle score group and increased gradually, with the highest triglyceride levels in the very-small-LDL particle score group. Patients with CAD had significantly higher triglyceride levels than did controls in the intermediate- and large-LDL score groups. Apo B levels were also lower in the large-LDL particle score group and increased gradually in the smaller LDL size range, similar to observations for triglyceride levels. Significant differences in apo B levels between CAD patients and controls were found in the intermediate-LDL particle score group. In contrast, higher ($p<0.0001$) HDL cholesterol and apo A-I levels were found in the large- and intermediate-LDL particle score groups compared with the small- and very-small-LDL particle score groups. It is important to note the significant differences in HDL cholesterol and apo A-I levels found between CAD patients and controls within the intermediate- and small-LDL particle groups. In both the intermediate- and small-

LDL particle score groups, patients with CAD had lower HDL cholesterol and apo A-I levels than did controls ($p<0.01$).

Low Density Lipoprotein Cholesterol Levels in Four Major Low Density Lipoprotein Particle Score Groups in Coronary Artery Disease Cases and Controls

The associations of LDL cholesterol and LDL particle score are different from those observed in other lipoprotein parameters. LDL cholesterol does not increase or decrease gradually with score. This is why no apparent association between LDL particle score and LDL cholesterol was detected in Table 2. In CAD patients and controls, LDL cholesterol levels were higher ($p<0.001$) in the intermediate- and small-LDL particle score groups than in the large- and very-small-LDL particle score groups. LDL cholesterol levels were higher in CAD patients than controls in all the LDL particle score groups except for the large-LDL particle score group, in which only 4% of CAD patients were found. Most men in both groups have intermediate LDL particles, but the lipoprotein parameters differ within this group, with CAD patients having significantly lower HDL cholesterol and apo A-I levels and higher triglycerides, LDL cholesterol, and apo B levels than controls.

Plasma Parameters That Best Discriminate Between Coronary Artery Disease Cases and Controls

Tables 5 and 6 show the parameters that best discriminate between CAD cases and controls. Because HDL cholesterol and apo A-I levels and LDL cholesterol and apo B levels are highly intercorrelated, we carried out two separate models: in the first one, we included HDL and LDL cholesterol but not apo A-I and apo B levels, and in the second one, we included apo A-I and apo B levels but not HDL and LDL cholesterol. Smoking was a strong discriminator in both models, where 67% of cases and 29% of controls smoked more than 10 cigarettes per day. Diabetes and hypertension were significant discriminators in these models as well. The prevalence of these risk factors was 12% and 42% among the cases and 3% and 12%

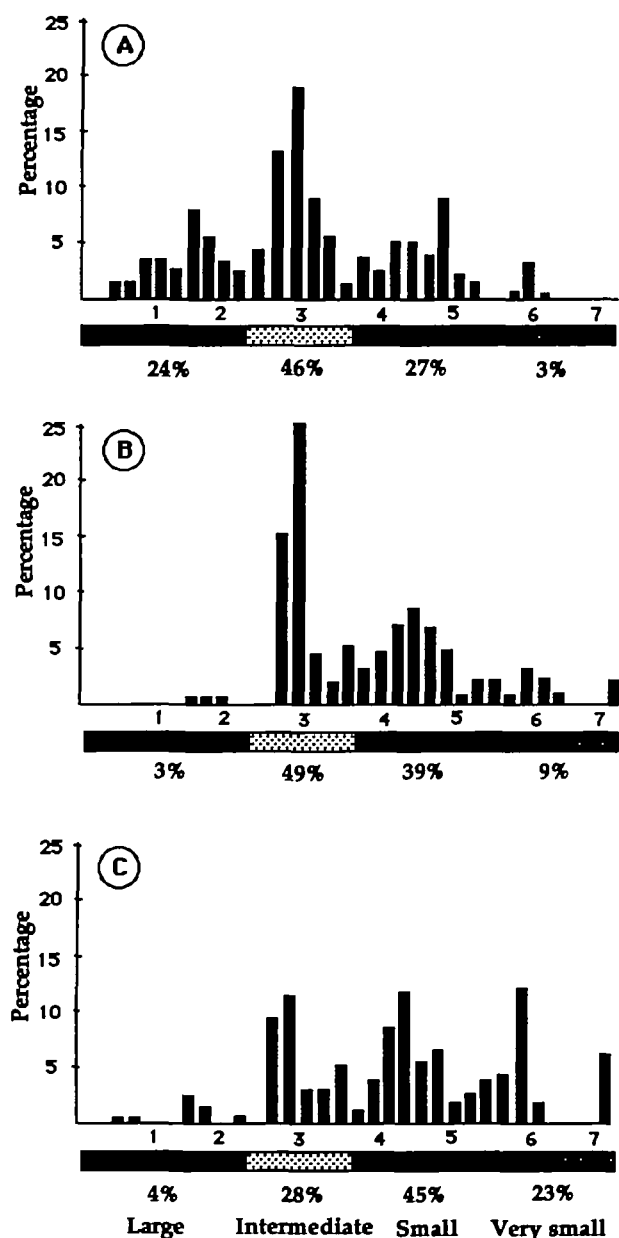


FIGURE 2. Bar graphs of low density lipoprotein (LDL) particle score distribution in (panel A) control group ($n=822$), (panel B) men with coronary artery disease off β -blockers ($n=96$), and (panel C) men with coronary artery disease on β -blockers ($n=179$).

among the controls, respectively. Of the biochemical risk factors, HDL cholesterol and LDL cholesterol levels or apo A-I and apo B levels remained as independent significant discriminators of CAD in these models. There was no indication from these data that apo A-I and apo B were substantially better discriminators of CAD risk than were HDL and LDL cholesterol. These analyses also show that triglyceride levels and LDL particle size were not independent risk factors. Because triglyceride levels and LDL particle size are also highly intercorrelated (Table 2), we entered them in the model independent of each other as well.

However, when either triglyceride or LDL particle size was entered alone, they still did not reach independent significance in the presence of the established risk factors that were in the model.

Discussion

Recent studies indicate that small, dense, low-molecular-weight LDL is associated with increased risk of CAD.^{14,15} It has also been suggested that polydisperse LDL is associated with atherosclerosis in hypertriglyceridemic diabetic subjects.²² In addition, the smaller, denser, apo B-rich LDL that characterizes patients with hyperapobetalipoproteinemia is a risk factor for CAD.^{13,23} In our study, we found an increased prevalence of small LDL particles in CAD patients compared with controls. In addition, only 3% of CAD cases had large LDL particles compared with 27% of controls. We did not find any association between CAD and LDL polydispersity, as has been previously reported.²²

LDL particle size has been proposed to be a heritable trait but one that is not fully expressed in premenopausal women and young men.²⁴ Our recent data indicate that there is only moderate heritability for LDL particle size in twins.²⁵ The factors previously found to be associated with decreased LDL particle size are male gender, increased triglyceride, very low density lipoprotein mass, intermediate density lipoprotein mass, apo B, and decreased HDL cholesterol and apo A-I levels.^{9,10,15,26,27} Thus, it has been suggested that LDL particle size is a marker for these series of metabolic alterations, which are probably influenced by similar mechanisms.²⁷ However, a series of environmental factors are highly associated with LDL particle size. A high prevalence of small LDL particles has been found in populations who consume low-fat, high-carbohydrate diets and who currently have a lower incidence of CAD than that in the United States.¹¹ In addition, LDL particle size is highly associated with total and abdominal fat in the same population.²⁸ Furthermore, LDL particle size is correlated with exercise in women.²⁹ In our present study, we examined dietary intake in a subset of 43 patients and 76 controls (data not shown). Dietary carbohydrate intake was significantly higher and dietary fat intake was significantly lower among CAD patients compared with controls. Thus, some of the differences in LDL particle size observed between CAD patients and controls in this study could be due to these differences in diet as well; elevated triglyceride and decreased HDL cholesterol were also associated with small LDL particles in our study. It has been proposed that elevated plasma triglyceride levels permit continued particle-size reduction through lipase action, as it might afford a substrate for the cholesteryl ester exchange protein as intermediate density lipoprotein and LDL become enriched in triglyceride and lose cholesteryl ester.³⁰

Another environmental factor associated with lipoprotein alterations is the use of medications such as β -adrenergic blockers.³¹ In the current studies, we

TABLE 4. Lipoprotein and Apolipoprotein Levels by Low Density Lipoprotein Particle Score Group* Distribution in Men With Coronary Artery Disease and Controls

LDL particle score group*/ analysis of variance	Subjects <i>n</i>	%	Triglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	Apo A-I (mg/dl)	Apo B (mg/dl)
Large LDL (≥ 1.00 , ≤ 2.60)							
Control	199	24	67 \pm 30†	136 \pm 32	49 \pm 12	132 \pm 36	86 \pm 23
CAD	11	4	137 \pm 73	128 \pm 56	55 \pm 15	147 \pm 41	80 \pm 29
Intermediate LDL (> 2.60 , ≤ 3.80)							
Control	376	46	116 \pm 43‡	141 \pm 34	42 \pm 9‡	130 \pm 28†	96 \pm 24‡
CAD	97	35	137 \pm 61	152 \pm 58	38 \pm 11	115 \pm 26	106 \pm 31
Small LDL (> 3.80 , ≤ 5.60)							
Control	220	27	201 \pm 81	136 \pm 31	38 \pm 8‡	125 \pm 29†	105 \pm 32
CAD	117	43	195 \pm 66	143 \pm 36	33 \pm 7	108 \pm 25	114 \pm 29
Very small LDL (> 5.60)							
Control	27	3	261 \pm 115	121 \pm 44	33 \pm 7	119 \pm 32§	105 \pm 41
CAD	50	18	289 \pm 92	140 \pm 31	29 \pm 5	101 \pm 18	117 \pm 24
LDL score <i>p</i> value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CAD vs. control group <i>p</i> value			0.02	0.01	<0.0001	<0.0001	0.002
β -Blocker use			0.3	0.006	0.06	0.1	0.04

LDL, low density lipoprotein; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; Apo, apolipoprotein; CAD, coronary artery disease.

Values are given as mean \pm SD adjusted for the effect of β -blocker use. Controls, *n*=822; CAD, *n*=275.

*LDL particle score for each subject was calculated by multiplying each LDL band present by its percent relative area. LDL particle score groups were assigned according to the LDL particle score population distribution found in this study (see text for details).

†*p*<0.0001, ‡*p*<0.01, §*p*<0.05; significantly different from CAD group within the same LDL particle score group.

report that CAD patients on β -blockers are significantly more likely to have small LDL particles compared with those not taking this medication. The magnitude of the difference between controls and CAD patients in LDL cholesterol was increased, and the difference in triglyceride, HDL cholesterol, apo A-I, and LDL particle size was reduced after adjusting for β -blocker use. In addition, our data also

indicate that the prevalence of the very small LDL is associated with the use of β -blockers. The prevalence of small and very small LDL found in CAD cases (48%) in our study is somewhat lower than the prevalence of pattern B in CAD cases (54%) in a recent report by Austin et al.¹⁵ We have estimated that small and very small LDL in our study corresponds to LDL size ≤ 260 Å, whereas pattern B (small LDL) has been defined as < 255 Å by Austin et al.¹⁵ These differences in cutpoint would not

TABLE 5. Discriminant Analysis for Coronary Artery Disease With High Density Lipoprotein and Low Density Lipoprotein Cholesterol but Not Apolipoprotein A-I or Apolipoprotein B

Variables entered	Partial <i>R</i> ²	<i>F</i> statistic	<i>p</i> value
β -Blocker use*			
Smoking habits†	0.04	49.0	<0.0001
Diabetes‡	0.03	39.8	<0.0001
Hypertension†	0.03	32.1	<0.0001
LDL cholesterol	0.02	26.5	<0.0001
HDL cholesterol	0.01	14.9	<0.0001
LDL particle size‡	0.00	0.9	NS
Triglyceride‡	0.00	0.1	NS
Total <i>R</i> ²	0.13		

Analysis includes all coronary artery disease patients (*n*=275) and controls (*n*=822).

LDL, low density lipoprotein; HDL, high density lipoprotein; NS, not significant.

* β -Blocker use was entered in the model to account for the effect of this medication on plasma lipoproteins.

†Smoking habits, diabetes, and hypertension are coded as 0=no or 1=yes.

‡LDL particle size (*F* statistic=6.9, *p*<0.01) or triglyceride (*F* statistic=5.5, *p*<0.05) was significant when HDL cholesterol was not in the model. Triglyceride was no longer significant in the presence of LDL particle size.

TABLE 6. Discriminant Analysis for Coronary Artery Disease With Apolipoprotein A-I and Apolipoprotein B but Not High Density Lipoprotein or Low Density Lipoprotein Cholesterol

Variables entered	Partial <i>R</i> ²	<i>F</i> statistic	<i>p</i> value
β -Blocker use*			
Smoking habits†	0.04	47.3	<0.0001
Diabetes‡	0.03	33.7	<0.0001
Hypertension†	0.03	31.0	<0.0001
Apo B	0.02	25.7	<0.0001
Apo A-I	0.02	24.5	<0.0001
Triglyceride‡	0.00	0.7	NS
LDL particle size‡	0.00	0.5	NS
Total <i>R</i> ²	0.14		

Analysis includes all coronary artery disease patients (*n*=275) and controls (*n*=822).

Apo, apolipoprotein; LDL, low density lipoprotein; NS, not significant.

* β -Blocker use was entered in the model to account for the effect of this medication on plasma lipoproteins.

†Smoking habits, diabetes, and hypertension are coded as 0=no or 1=yes.

‡Triglyceride and LDL particle size did not reach statistical significance even when apo A-I was not in the model.

explain the slightly higher prevalence of small LDL observed by Austin et al.¹⁵ The difference is probably due to the fact that in the prior study, CAD patients taking β -blockers were not excluded. It should also be noted that the end point previously reported was myocardial infarction, as opposed to coronary angiography in the present study. Moreover, the prevalence of small and very small LDL in controls (30%) in our study was very similar to the prevalence of pattern B previously reported for the control group (31%) by Austin et al.¹⁵

In previous studies comparing LDL particle size of patients with CAD and controls,^{14,15} the LDL particle size differences between these two groups were reduced to nonsignificance after adjusting for triglyceride levels. Our data indicate that smoking, hypertension, diabetes, and HDL and LDL cholesterol were strong discriminators of CAD, whereas triglyceride and LDL particle size were not independently associated with CAD. Thus, small LDL particles and triglycerides are not independent risk factors; rather, their association with low HDL cholesterol suggests that these parameters may reflect a series of alterations in lipoprotein metabolism that increase CAD risk. The question remains whether small LDL particles per se are atherogenic. A recent study indicates that smaller, denser LDL particles from normal plasma are more susceptible to oxidation in vitro.³² In contrast, cholesterol-fed monkeys have large cholesteryl ester-enriched LDL, which deliver more cholesteryl ester per particle to cells in the arterial wall and are positively associated with atherosclerosis.³³ Furthermore, large LDL particles contain more saturated cholesteryl esters in a liquid crystalline state at body temperature, and it has been suggested that LDL particles with such cores are more atherogenic.^{34,35} Most likely it is not only size but also a series of physical and chemical characteristics of LDL that are relevant in determining its atherogenicity in humans.

Apo A-I and apo B concentrations have been associated with the presence of CAD and have been proposed as better discriminators than HDL cholesterol or LDL cholesterol.³⁶⁻³⁹ We did not find an indication that apo A-I and apo B were substantially better discriminators of CAD risk than were HDL and LDL cholesterol. In agreement with our study, it has recently been reported that protein-enriched LDL was not found to be a risk factor for CAD after adjusting for age, smoking, and weight⁴⁰ and that the ratio of total to HDL cholesterol was the best biochemical predictor of myocardial infarction in a prospective study, with no other significant variables in the model.⁴¹

In sum, β -blockers are associated with a reduced prevalence of intermediate LDL particles and increased prevalence of small and very small LDL. Large LDL particles are absent and small LDL particles are more prevalent in CAD patients compared with controls. Small LDL particles are not independently associated with CAD after other es-

tablished risk factors such as smoking, hypertension, diabetes, and lipoprotein parameters such as LDL and HDL cholesterol have been taken into account.

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